**How berry enlargement practices affect the flavor of grapes**

Lichter, A. Agricultural Research Organization
Lewinsohn, E. Agricultural Research Organization
Ebeler, S.E. The Regents of the University of California, Davis

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**Abstract**

Berry enlargement in seedless grapes is carried out by the plant growth regulators (PGRs) gibberellic acid (GA), cytokinin (in the form of CPPU) and by trunk girdling. The objective of the research project was to understand how these treatments influence metabolic processes with emphasis on the phenylpropanoid pathway and flavor components. The treatments were applied at berry size of 6 mm diameter on the black and aromatic cultivar 'Sable Seedless', on the green cultivar 'Superior' and on the wine grape 'Sangiovese' that due to the presence of seeds, is not supposed to be enlarged by PGRs (girdling was not performed on the wine grape). The PGR treatments on 'Sable' increased berry size, delayed sugar accumulation and acid degradation with a stronger effect of CPPU. While CPPU reduced the levels of anthocyanins by more than 50%, the combined treatment of GA+CPPU reduced the levels by only 25%, 51 d after application. The CPPU treatment had minor effects on flavonols content but increased the levels of monomeric flavan-3-ols by more than two-fold. Phloroglucinol analysis using HPLC showed that proanthocyanidin content was significantly increased by CPPU whereas mean degree of polymerization was reduced from 26 to 19. Volatile analysis by GC-MS showed changes in composition with CPPU or GA treatment with potential impact on flavor. RNA-seq analysis showed that GA had a minor overall effect on the transcriptome whereas CPPU had pronounced effects on gene expression at both 51 and 70 d. Comparing the control and CPPU at similar Brix of ca. 19.7°, a reduced expression of *stilbene synthases* (STSs) including their regulators *MYB14* and *MYB15*, and other phenylpropanoid-related genes was observed in CPPU treated grapes. PGRs treatment on 'Sangiovese' significantly increased berry diameter and berry weight and the CPPU containing treatments increased skin thickness. All treatments reduced sugar accumulation and color development but did not have an effect on acid levels. HPLC analysis showed that total flavan-3-ols level was increased by CPPU, GA containing treatments reduced the levels of flavonols and that anthocyanins were significantly reduced in all the treatment (≈50%). Phloroglucinol analysis showed that proanthocyanidins level were significantly increased whereas the mean degree of polymerization was reduced from 40 to ≈21 by all the treatments. GC-MS analysis of berries showed a decrease in fatty acid derived volatile compounds by all treatments with potential impact on flavor. Analysis of wine phenolics in subsequent harvest was in general agreement with the previous results. In girdling there is a temporary cessation of sugar allocation to the roots making the developing berries a primary sink. Girdling was performed on Superior Seedless Sable Seedless. As expected, girdling resulted in consistent increase in berry size but total soluble content of mature 'Superior' berries was not affected and in 'Sable' it was slightly reduced in one of the two seasons examined. One week after girdling, abscisic acid and gibberellin content was higher in fruitlets from girdled vines and genes of the phenylpropanoid pathway were induced in both cultivars. Berry color development of 'Sable' was reduced upon girdling. In contrast, flavan-3-ol and flavonol content, and total proanthcyanidins (PA) content increased 1.8-fold while the mean degree of polymerization of the PA decreased from 26 to 21 upon girdling. Girdling reduced the levels of fatty acid derived volatiles in berries of 'Superior' and 'Sable'. In 'Sable', the total terpene level and the level of volatiles released after acid hydrolysis, decreased upon girdling. Overall, our study indicates that girdling can divert metabolic pathways in a manner that may have a significant effect on the taste and flavor of grapes.

## Summary Sheet

## Publication Summary

| PubType | IS only | Joint | US only |
|---|---|---|---|
| Other | 0 | 1 | 0 |
| Reviewed | 0 | 2 | 0 |
| Submitted | 0 | 2 | 0 |

## Training Summary

| Trainee Type | Last Name | First Name | Institution | Country |
|---|---|---|---|---|
| Postdoctoral Fellow | Tyagi | Kamal | Volcani, Davis | Israel |
| Postdoctoral Fellow | Maoz | Itay | Davis, Volcani | USA |

**Contribution of the collaboration**

The vineyard experiments were all carried out in Israel including phenology, sampling and some of the GC-MS analyses. Samples of the 2018 season were sent to the lab in Davis. A student from Israel (Itay Maoz) tried during the summer of 2018 to further develop the Q-TOF methodology for analysis of long-chain tannins but he reached a glass-sealing of about 14-mer polymers while polymer length in the berries were much larger and therefore this method had to be abandoned for the project. In early 2019 Dr Kamal Tyagi prepared RNA samples in Israel that were later sent for sequencing using a unique platform developed by the core facility in Davis and which permitted the analysis of relatively short RNA molecules in a significantly better cost-effective manner. The sequencing data was analyzed by a bionformatic expert in Israel. In 2019 Dr Tyagi went to Davis to extract and analyze the phenolic monomers and tannins by purification and HPLC methodology developed in Davis. In 2020 Dr Tyagi returned to Israel and he established another field experiment on Sangiovese vines. The grapes from this experiment were processed into wine which he analyzed by the same methodologies he learned in Davis. On another token, Dr Tyagi wrote a book chapter on methodologies used in the project together with the partners in Davis and together with an expert in Italy (Dr Flamini) with which the Israeli PI had past collaboration. All 5 papers generated by the project were written by the partners in Israel and Davis. Traveling of the Israeli PI was planned in the autumn of 2019 but was delayed due to the request to extend the project and eventually could not be done due to COVID-'20' issues.

**Achievements**

Enlargement of berries of table grapes is a key technology in table grape production. These techniques are used to increase the yield for the growers by substantial amount. Consider a 20% increase in size of individual berries as a 20% increase in yield. This requires that the treatments do not damage the quality of the cluster by making it too tight or by reducing the color and sugar, increasing astringency (dry mouth feel) or causing other metabolic changes that can reduce the flavor or health related properties. On the other hand, delay in ripening can be either beneficial or unwanted depending on marketing issues. We took the task to understand these issues by applying 3 major technologies, application of the plant growth regulators, gibberellin, cytokinin and girdling to 2 table grape cultivars and one wine grape cultivar. Gibberellin is the most widely used technique for 70 years, cytokinin was introduced about 30 years ago and girdling is also a traditional technique whereby the vessels that carry sugar from the leaves to the roots are temporarily disconnected with a special knife thereby allowing more sugars to flow to the berries at a critical time for their development. Our research found that the treatments had the expected results on berry size and delay in maturation for some of the treatments and this was a prerequisite for successful research. At the anatomical level, we found that all treatments increased slightly the thickness of the skin which is good for protection against diseases both in the vineyard and after harvest. In the black colored cultivar 'Sable', girdling caused diversion of a major pathway that leads to synthesis of red pigments to polymers called tannins that are responsible for astringency including reduction in the length of the polymers. The treatment also reduced the level of volatiles responsible for the flavor of the grapes, potentially due to delayed ripening. Gene expression one week after the treatment suggested that part of the pathway responsible for the synthesis of these compounds was induced. With respect to gibberellin and cytokinin we found dramatic effects of the cytokinin on many important phenolic compounds but no or minor effect of gibberellin on these compounds. Along with the increase in tannins by cytokinin, there was a major effect on a particular branch of the pathway that is responsible for synthesis of compounds that are considered to have health benefits. This finding was corroborated by the fact that the gene family responsible for the synthesis of these compounds, as well as the proteins that specifically regulate this gene family, had lower expression. Another objective of this study was to understand how gibberellin and cytokinin affected wine grapes that are not subject to such treatments mainly because there is no incentives to increase yield and because these are seeded grapes that are less affected by growth regulators. However, we wanted to test if the treatments had an effect on the tannin content which could have improved the wine quality. We did find that tannins were increased by cytokinin in Sangiovese grapes that suffer from relatively "light body" as winemakers describe it. In contrast to table grapes, an increase in tannins in wine grapes can be a positive outcome so these results may promote further applied research to determine if the positive effect on tannins is not counteracted by a delay in ripening. Gibberellin had some positive effects on berry quality but it is not yet clear if it is justified to apply it. It can be concluded that girdling and cytokinin treatments can have negative effects on table grape quality and their application should be carefully considered. In contrast, gibberellin seems to be a 'natural' treatment for grapes that, if used as recommended, does not have adverse effects on the quality of grapes.

**Changes to the original research plan**

The original objectives were:

1) Study the effect of cytokinin, gibberellin and girdling on metabolites of the phenylpropanoid pathway
2) Determine the effect of cytokinin, gibberellin and girdling on the free and bound volatile composition of grapes
3) Study the effect of cytokinin, gibberellin and girdling on genes associated with flavor aspects of the phenylpropanoid pathway
4) Determine if girdling changes the growth regulator balance in the berry
5) Study how the treatments affect the histochemical and microscopic properties of the cuticle
6) Study what are the consequences of the cultural practices the flavor quality of 'neutral' and 'aromatic' grapes.

All objectives were met with some changes to the original workplan

- Changes were carried out between 2017 and 2018 in the sampling methodology and therefore samples collected in 2017 were not used for analytical purposes.
- In 2018 it was not possible to repeat the experiment on Superior grapes due to horticultural problems in the vineyard.
- Itay Maoz traveled to Davis in the summer of 2018 to further develop the Q-TOF methodology to fit for the table grape samples but eventually it was not possible to use this technique due to limitations of the polymer sizes and the complexity of permutations of the monomers. Therefore, Dr Tyagi used the phloroglucinol methodology according to a protocol developed by another laboratory in Davis.
- Due to scientific considerations, sampling for RNA sequencing of berries from girdled vines was done only after 1 week from girdling. Sampling of GA and CPPU treated grapes for RNA sequencing was eventually done after veraison and at harvest in order to understand the long-term effects of the treatments. Methodology was in part different from that proposed due to the development of new platforms for sequencing and data analysis.
- In the original work plan Sangiovese wine was planned to be made after applying the different treatments in Davis but because samples from 2018 were analyzed only in 2019, it was not possible to reach a decision if to do the experiment in 2019. Therefore, an extension to the project was requested and the wine was prepared and analyzed in Israel.
- Postharvest experiments on the treated grapes was eventually not carried out.
- The traveling budget of Dr Ebeler was dedicated to support the travel of Dr Maoz.

## Publications for Project IS-4957-16 R

| Status | Type | Authors | Title | Journal | Vol:pg Year | Coun |
|---|---|---|---|---|---|---|
| Published | Reviewed | *Tyagi, K., Maoz, I., Lewinsohn, E., Lerno, L., Ebeler, S.E., Lichter, A.* | Girdling of table grapes at fruit set can divert the phenylpropanoid pathway towards accumulation of proanthocyanidins and change the volatile composition | ***Plant Science*** | 296 : 13 2020 | Joint |
| Published | Reviewed | *Tyagi, K., Maoz, I., Kochanek, B., Sela, N., Lerno, L., Ebeler, S.E., Lichter, A.* | Cytokinin but not gibberellin application had major impact on the phenylpropanoid pathway in grape | ***Horticulture Research*** | 8:51 : 15 2021 | Joint |
| Published | Other | *Lichter, A., Tyagi, K., Maoz, I., Carmeli-Weisberg, M., Shaya, F., Teitel, Z., Lerno, L., and Ebeler, S.* | Al Yithalel Choger Kemefateach": The impacts of girdling on flavor components in grapes | ***Hanotea*** | 74 : 46-50 2020 | Joint |
| Submitted | Book Chapter | *Tyagi, K., Lerno, L., De Rosso, M., Maoz, I., Lichter, A, Ebeler, S.E., Flamini, R.* | Extraction and analysis of phenolic compounds from grape berries | ***Plant secondary metabolism engineering: methods and protocols*** | : 15 2021 | Joint |
| Submitted | Reviewed | *Kamal Tyagi, Itay Maoz, Or Lapidot, Bettina Kochanek, Meir Shlisel, Larry Lerno, Susan E. Ebeler, Amnon Lichter* | The effects of gibberellin and cytokinin treatments after berry set on the phenolic and volatile composition in Vitis vinifera cv. Sangiovese grapes | ***not yet*** | : 24 2021 | Joint |